

# The first recorded occurrence of the Asian hornet (*Vespa velutina*) in Ireland, genetic evidence for a continued single invasion across Europe

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## Abstract

The first record of the Asian/yellow-legged hornet (*Vespa velutina*) in Ireland was reported in April 2021, when a single female individual was discovered in Dublin. *Vespa velutina* has been present in mainland Europe since 2004 and in the UK since 2016 and poses an enormous threat to European apiculture and bee-mediated pollination services. Three mitochondrial genes were sequenced from the Irish specimen to determine whether the specimen originated from the established European population or signified a new point of entry from its native range in China. Additionally, specimens from Portugal, Spain, France, Germany, and the Channel Islands were sequenced at these three genes to build on previous studies which have asserted, based solely on Cytochrome Oxidase 1 (COI) analysis, that the entire range of *V. velutina* in Europe represents a single invasion which has proliferated since the first record in France. Further data were retrieved from GenBank for comparison. Results reveal that the mtDNA lineage observed in Dublin is the same as that seen throughout Europe, and therefore the arrival of this species in Ireland likely represents a further spread of the ongoing European invasion.

## Keywords

Asian hornet, haplotype, invasive, Ireland, mtDNA

## Introduction

The discovery of an Asian hornet, also known as the yellow-legged hornet (*Vespa velutina*, Lepeletier, 1836) in Dublin in April 2021 (<https://biodiversityireland.ie/asian-hornet-alert/>) has raised concern among beekeepers and biologists in Ireland due to the threat posed to apiculture through predation on domestic European honey bees (*Apis mellifera*) and other important pollinators (Monceau et al. 2014). *V. velutina* is native to South-East Asia, where it hunts a range of pollinating insects, and has been observed, when introduced, to be extremely successful in colonising new areas (e.g. Villemant et al. 2011; Monceau et al. 2014; Arca et al. 2015). Since the first record of *V. velutina* in France in 2004 it has become widespread throughout continental Europe and Jersey Island (Lopez et al. 2011; Villemant et al. 2011; Monceau et al. 2014; Arca et al. 2015; Robinet et al. 2016; Robinet et al. 2018; Husemann et al. 2020a; Laurino et al. 2020) while in England there have been 21 confirmed sightings (12 nests) since 2016 - (<https://www.gov.uk/government/publications/asian-hornet-uk-sightings/asian-hornet-sightings-2020>). Given that the species is expanding its range at an estimated 75 and 82 km/year in mainland Europe (Robinet et al. 2016), it is critical from an Irish perspective to determine the path of this potential invasion and gain an understanding of dispersal dynamics.

Previous studies addressing the provenance of Asian hornets in Europe have focused on the Cytochrome oxidase subunit I (COI or COX1) gene within the mitochondrial DNA (mtDNA) genome. However, the reliance on a single, relatively slow evolving/mutating gene may result in an oversimplification of invasion dynamics. Jeong et al. (2021) considered this and utilised sequence data from four other genes to track the invasion of Asian Hornets in South Korea. Analysing specimens from 11 Korean and 2 Japanese localities revealed that all individuals had identical sequences at COI, CytB (cytochrome B) and 16S rRNA (16S rRNA), which could have been interpreted as a single invasion. However, two intergenic spacer (IGS) sequences displayed substantially more variability indicative of multiple entry sites for *V. velutina*, independent of the southeast region, which had previously been considered as the sole entry point of the invasion.

We analysed sequences at three mtDNA genes; COI, IGS2 and IGS3 in the specimen recovered from Dublin, Ireland. Due to a paucity of sequence data (other than COI) for Asian hornets in Europe, we also sought samples from continental Europe and the island of Jersey, with which to compare data from this ‘Irish’ hornet. We further sourced available sequence data from GenBank for comparison.

## Materials and methods

### Samples and laboratory analysis

The *V. velutina* specimen was deposited with the entomological collections in the National Museum of Ireland (specimen number NMINH:2021.2.1) immediately after its discovery. A single middle leg was removed from the pinned specimen (Fig. 1) and



**Figure 1.** Female *V. velutina* specimen from Dublin, Ireland (museum specimen no. NMINH:2021.2.1). Note the absence of the middle left leg, which was removed below the coxa for DNA extraction.

stored in ethanol for subsequent DNA extraction. Additional whole specimens from across Europe were provided by researchers from Portugal (4), Spain (3), France (3), Germany (3), and Jersey Island (3).

DNA extraction was carried out using DNeasy Blood and Tissue Kit (Qiagen). Three mtDNA genes were chosen for analysis; cytochrome oxidase I (COI), Intergenic spacer region 2 (IGS2) which spans *COII-trnK-trnD-ATP8* and Intergenic spacer region 3 (IGS3) spanning *trnR-trnN-trnE-trnS<sub>1</sub>-trnF*. COI primers were from Folmer et al. (1994) and amplified a product of 707 bp. Primers for IGS1 and IGS2 were from Jeong et al. (2021) and these amplified products of approximately 390 bp and 700 bp respectively.

PCRs were performed in 20 µl volumes consisting of 10 µl of 2× Plain Combi PP Master Mix (Top-Bio), 1 µM each of forward and reverse primers and 10–50 ng of DNA. PCR cycling conditions were as follows; an initial denaturation step of 3 minutes at 95 °C was followed by 40 cycles of 95 °C for 30 seconds, 48 °C for 30 seconds and 72 °C for 1 minute, with final extension step of 72 °C for 5 minutes. Electrophoresis of PCR products was performed on 1% agarose and products were excised and purified using a QIAquick Gel Extraction Kit (QIAGEN). Sequencing was performed with the forward primer for each locus using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were purified using the EDTA-ethanol precipitation method described in the sequencing kit handbook and were run on an ABI3500XL DNA analyser.

Raw sequence data was examined using Chromas software 2.6.6.(Technelysium Pty Ltd) to resolve ambiguous calls. 5' and 3' ends of sequences were trimmed to the first shared nucleotides as gaps in sequences would be incorrectly interpreted as genetic distance during analysis rather than nucleotides that failed to be sequenced.

## Sequence analysis

All sequences were used in BLAST searches which served two purposes; firstly to confirm species and secondly so that search results could be downloaded for downstream alignments and analysis.

Each locus was analysed separately. Sequences were trimmed at either end to the point where full alignments could be performed and compared with publicly available data from other studies. Alignments were performed using the Clustal Omega multiple sequence alignment tool (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) to determine whether the Dublin specimen was of the same sequence composition as those we analysed from around Europe, and also to compare with sequence data available on GenBank from other studies

## Results

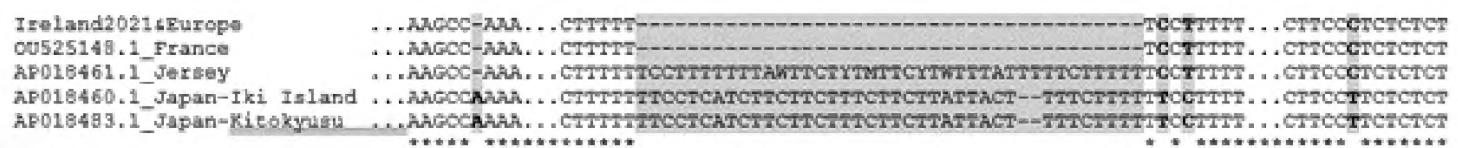
Sequencing of all three genes was successful for *the V. velutina specimen* discovered in Ireland. Success varied for the additional samples provided from Europe, but in all cases at least one hornet from each country was sequenced at each gene.

### COI

For COI, 557 bp of sequence were resolved for at least one individual from each area sampled. This sequence was identical in all of the specimens we examined. The sequence from the Dublin hornet was deposited on GenBank (Accession number OP437698). Using this sequence as a BLAST query revealed that this individual was also identical to all other sequenced and deposited samples from previous studies on Asian hornets in Europe, and therefore, like those, is most closely related to *V. velutina* inhabiting eastern China.

### IGS2

In the case of IGS2, we resolved 435 bp of sequence in the Dublin specimen (GenBank accession number OP537231). This was also identical across all of the European origin specimens we sequenced. There are only two other available sequences for this gene in Europe, one from France (OU525148.1) and another from the Channel Islands (AP018461.1, Takahashi et al. 2019), both of which were also 100% match (both of these sequences formed part of full mtDNA genome sequences). There are upwards of 200 other sequences from studies in Asia (native and non-native range) which differ from the European specimens, mostly in a short tandem repeat (AT) region. The number of repeats observed in this region varied from four (in all European specimens examined) to between 5 and 13 in Asia (Korea, Japan, Indonesia, China). Two other point mutations (SNPs) were also observed between European samples and the Asian range.



**Figure 2.** Summary figure of polymorphisms observed among IGS3 sequences. ‘...’ denotes areas of homologous sequence not included for brevity (AP018461, AP018460, AP018483 from Takehashi et al 2019; OU525148 from *Vespa velutina* genome assembly – sample provided by Seirian Sumner in collaboration with the Sanger 25 Genomes Project and Vertebrate Genomes Project (<http://vertebrategenomesproject.org>)).

IGS3

The IGS3 locus was successfully sequenced to a length of 700 bp in the Dublin specimen (GenBank accession number OP537232) and from all countries that we looked at. This sequence was identical in all hornets provided for this investigation. As with IGS2, there were just two sequences available from Europe for comparison (from the same studies). The French sample was identical to our specimens at this gene, but the Channel Islands sequence differed significantly in that there was a region of 37 bp present therein which was not seen in any of our European samples (Fig. 2), and that region was consistent with the Iki Island sample which was sequenced in that study (Takehashi et al 2019). There were however three additional single nucleotide polymorphisms and one single base pair deletion at which the Jersey Island sample was consistent with European samples and different from Iki Island. In the context of sequences available from throughout Asia (approximately 60), the 37 bp region in question was present in all cases, suggesting that the source population for Asian hornets in Europe has not yet been fully sequenced.

Discussion

Our analysis of three mtDNA loci is more comprehensive than has been previously undertaken within the range of invasive *V. veluntina* in Europe and suggests firstly that contemporary populations of Asian hornets in Europe are of a single phylogenetic lineage, and secondly that the Irish specimen is likely to have found its way (anthropogenically) to Dublin from Europe or Britain . Our study builds on earlier work (e.g. Arca et al. 2015; Budge et al. 2017; Granato et al. 2019; Husemann et al. 2020b; Jones et al. 2020; Quaresma et al. 2022) which utilised the cytochrome oxidase I gene and microsatellites to demonstrate a single invasion of *V.velutina* to Europe. The inclusion of additional mtDNA loci described in Jeong et al (2021) further indicates that, unlike the situation in Japan and Korea where multiple points of entry were detected, contemporary (2021) Asian hornet specimens collected across Europe, and the individual discovered in Ireland represent a common invasion history for hornets thus-far discovered in Europe, Britain and Ireland.

COI in our study specimens matched all available European sequences from other studies (Arca et al. 2015; Budge et al. 2017; Granato et al. 2019; Husemann et al. 2020b). In the case of IGS2 and IGS3, only two specimens in Europe had previously



been sequenced, one specimen from France (OU525148.1) which was identical in composition, and another from Jersey (Channel Islands, AP018461.1, Takehashi et al. 2019), which was characterised by a longer sequence length with a 37 bp insertion within IGS3, consistent with specimens from Japan and Korea (Jeong et al 2021), and indeed all previously sequenced samples from Asia currently available on GenBank. This specimen had been collected in Jersey Island in 2017. It would be worthwhile to analyse more individuals from a wider range of locations and dates particularly in the Channel Islands, France and the UK to determine if this polymorphism might be detected again.

The finding that the Irish specimen and those studied from Europe are likely to represent a single invasion (founder) event is not unexpected. Arca et al (2015) looked at microsatellite DNA alongside COI haplotypes and found that the French (and by extension European) invasion was descended from a population of *V. velutina* from eastern China, and that the introduction of a single queen fertilized by several males was the most likely scenario. Studies of nests discovered in Britain (Budge et al (2017), Jones et al (2020)) found even lower levels of genetic variability than had been observed in French and Korean specimens by Arca et al. (2015) with each nest containing only a subset of the alleles seen in French hornets, suggesting that incursions from mainland Europe are responsible for the presence of Asian hornets in England. We conclude that the discovery of the hornet in Dublin is also the result of an incursion from either mainland Europe or Britain, rather than an independent invasion from a source within the native East Asian distribution of this species. Further sequencing of individuals from the Zhejiang and Yunnan areas of Eastern China would be useful to determine if the IGS3 haplotype observed in Europe is present there, which would go further towards confirming whether that region was indeed the source of the European invasion.

Given the proximity of Ireland to Britain and continental Europe, and the existing trade links, it is prudent to expect further occasional anthropogenic introductions of *V. velutina*. Whether the species succeeds in establishing a self-sustaining population in Ireland is less certain. It is possible that climatic conditions in Ireland may prevent the establishment of a viable population, however the recent arrival, for example, of the Saxon wasp (*Dolichovespula saxonica* (Fabricius, 1793)) in the East of Ireland (Finch and Finch 2020) demonstrates the possibility of non-native social Vespidae to reach Ireland naturally from established populations in Britain, and highlights the need for vigilance from Irish authorities around the situation with *V. velutina*.

Finally, our results, along with those of other groups, suggest that the entire population of *V. velutina* in Europe, now potentially numbering many millions of individuals, are descended from a single mated queen arriving from China some 15–20 years ago. This demonstrates the potential for alien insects to become invasive pests via accidental imports of only very few, or single, individuals, and also the potential for biological control mechanisms, given the very low genetic diversity inherent in such populations.

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